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WS KRKP RKKR QL

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## MKKNIAFLLKR

STII Leader Sequence

(1:0H II DES)

## DSWMEEVIKLCGRELVRAQIAICGMSTWS B-Chain (SEQ 19 80:2)

Mini C peptide A-Chain Trypsin Trypsin CPB **QLYSALANKCCHVGCTKRSLARFC** KRKPTGYGSRKKR Trypsin (seq id no:4) (SEQ 1D 10:3)

**B-CHAIN** 

SIE

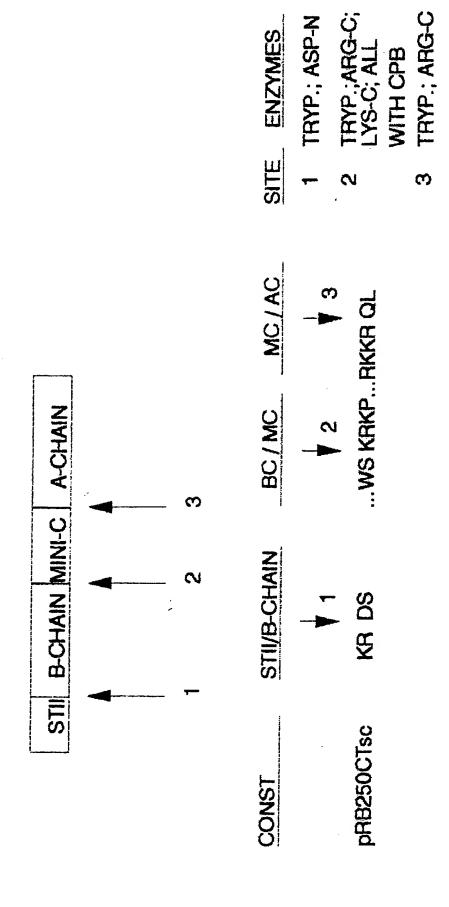
MINIC

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A-CHAIN

pRB 250 CT ac

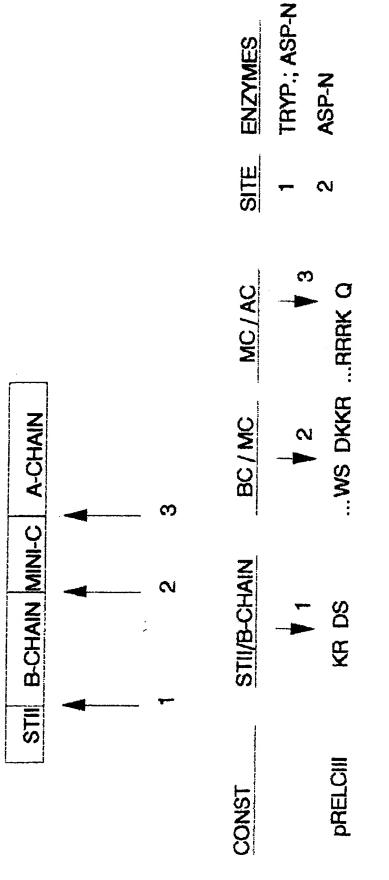
Plasmid Name	Leader Sequence	C chain sequence	Cleaving Enzyems
pRB250CTsc	MKKNIAFLKR	Trypsii KRKPTGYGSRKKR % (CPB)	Trypsin and carboxy-peptidase C (CPB)
pRELCIII	MICHIAFLUR	DKKRTGYGSRRRK 6	AspN and LysC
pRELCASPN	MONIAPLIKE	DKKRTGYGSRKKR 7	aspN and trypsin or AraC
PRELCLYSC	MICHIARLICE	KRKPTGYGSRRRK 8	LysC and CPB



MOST FAVORABLE COMBINATIONS

ARG-C / CPB TRYPSIN / CPB

FIG. 2A

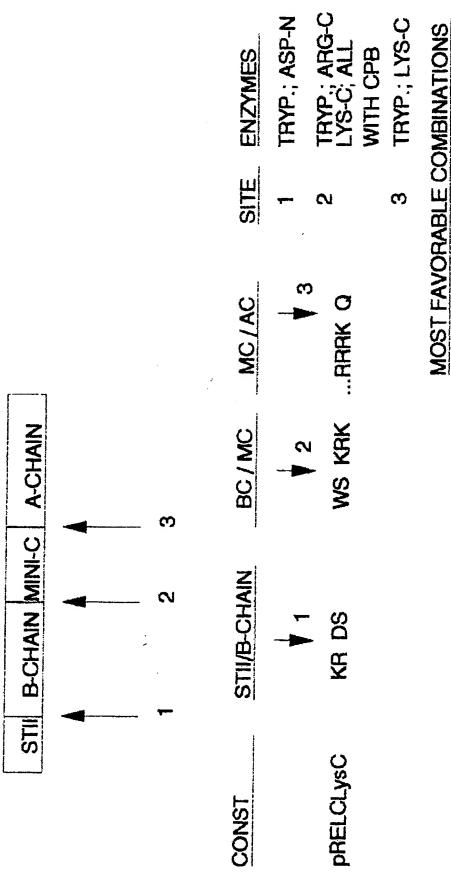


3 TRYP.; LYS-C

## MOST FAVORABLE COMBINATIONS ASP-N / LYS-C

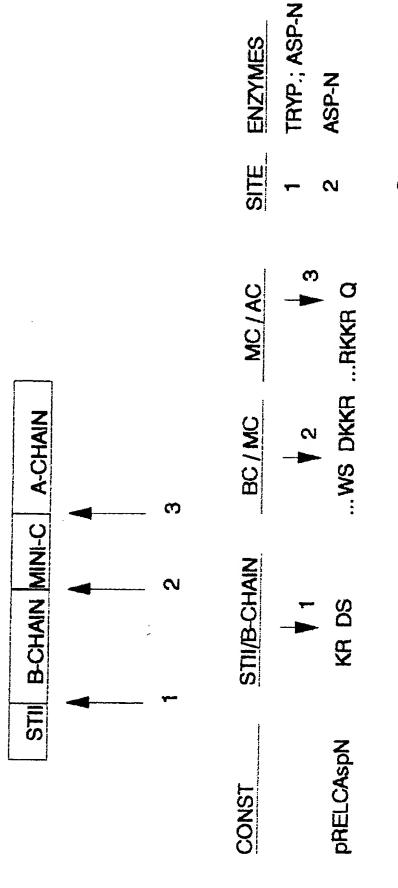
ASP-N / LYS-C ASP-N / TRYPSIN

FIG. 2B



TRYPSIN / CPB
TRYPSIN / LYS-C / CPB

TRYPSIN / LYS-C / CPB ARG-C / LYS-C / CPB



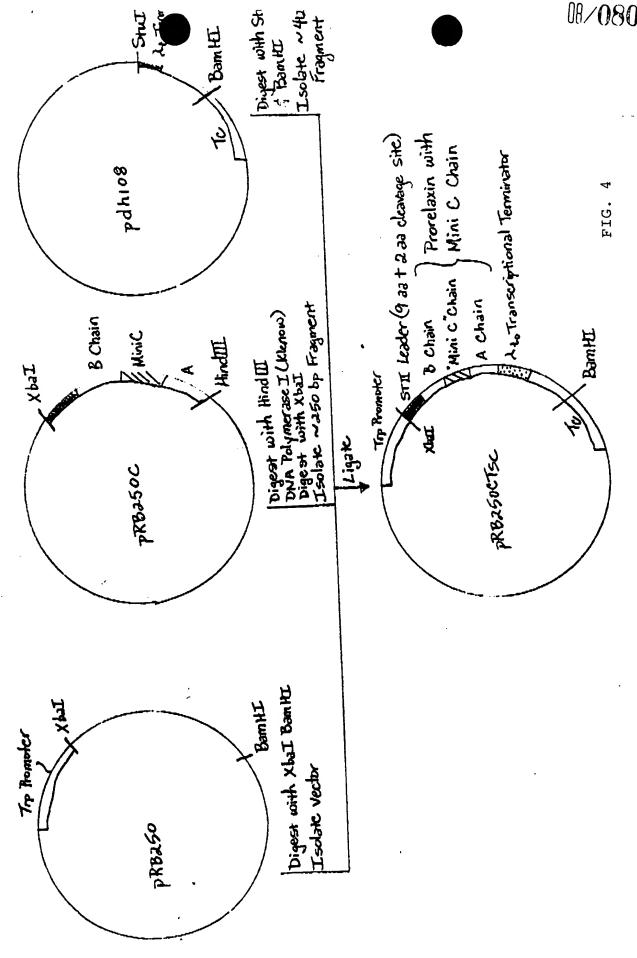
TRYP.; ARG-C ෆ

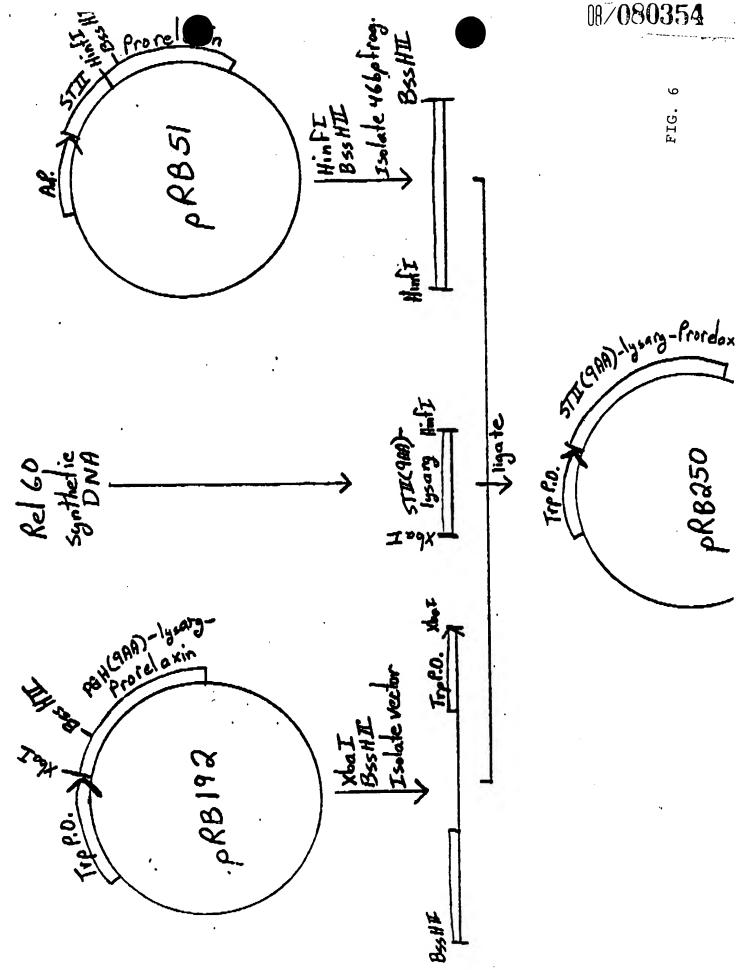
## MOST FAVORABLE COMBINATIONS ASP-N / ARG-C

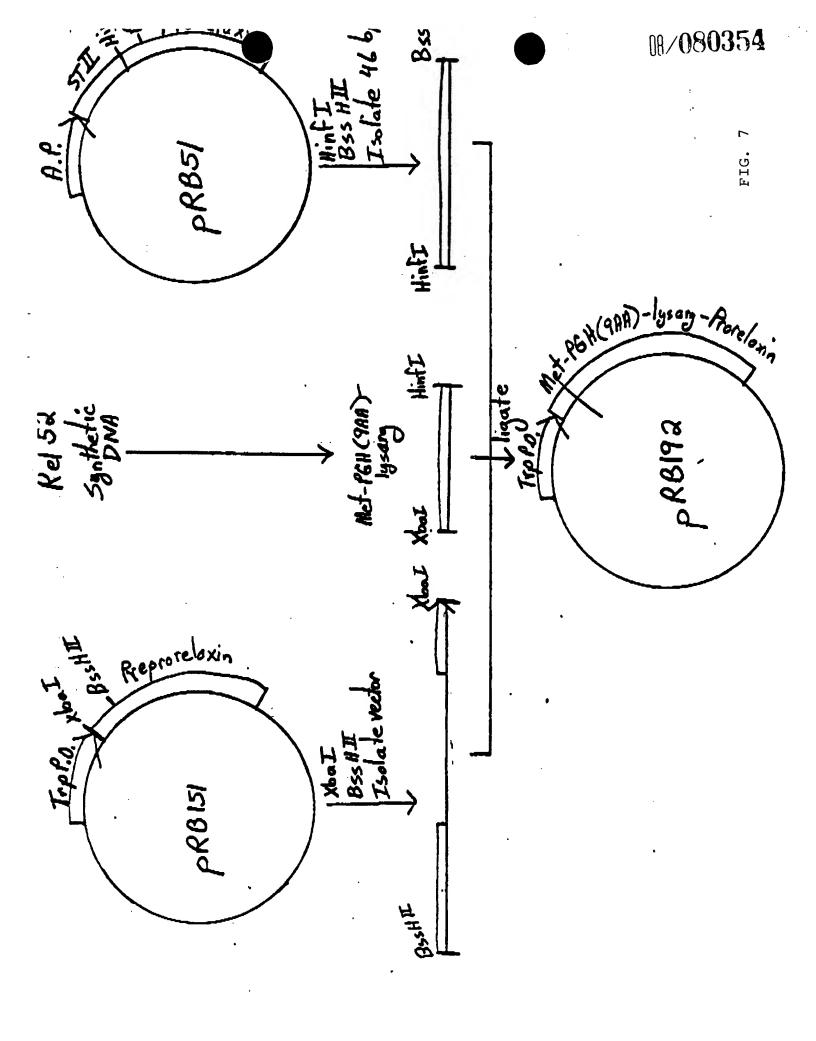
ASP-N / TRYPSIN

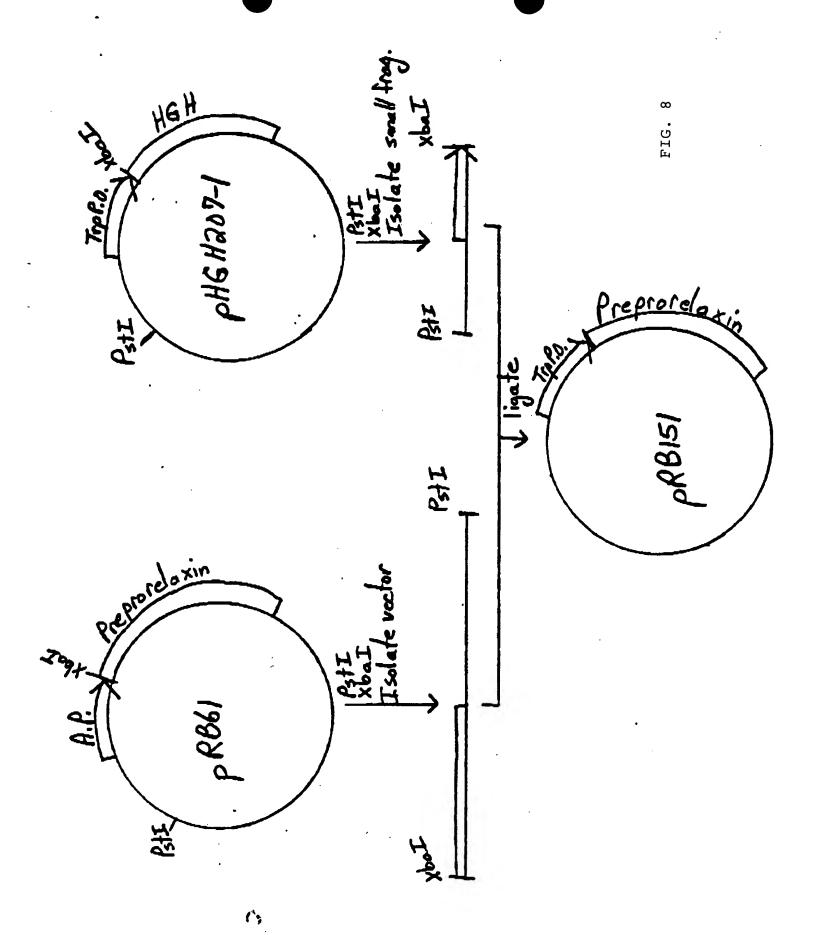
FIG. 2D

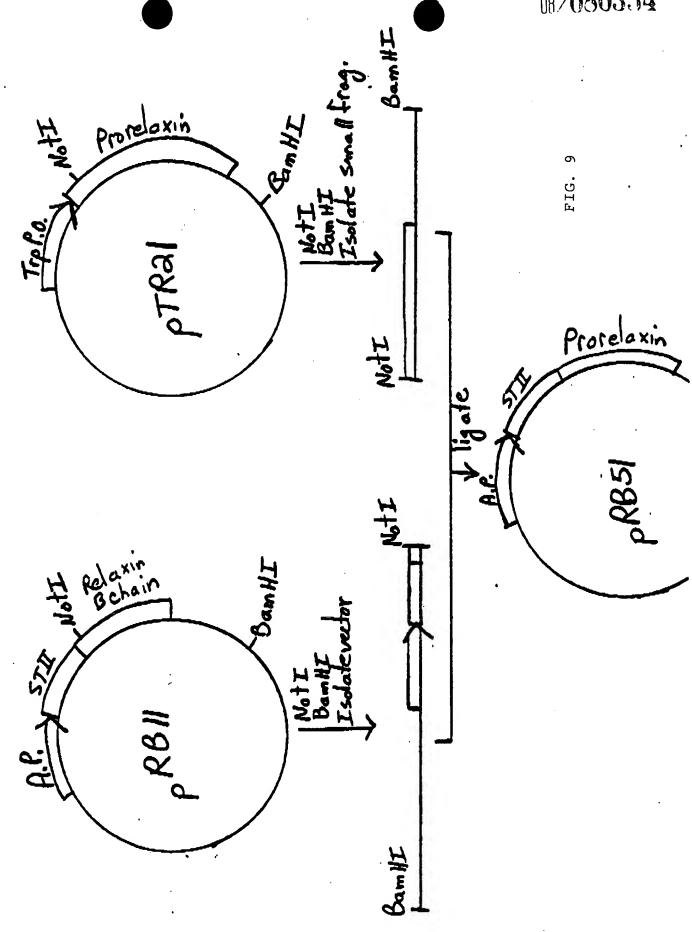
ATG AAA AAG AAT ATC GCA TIT CTT CTT AAA GGG GAC TCA 36 Met Lys Lys Asn Ile Ala Phe Leu Leu Lys Arg Asp Ser 1 STII leader + KR . TGG ATG GAG GAA GTT ATT AAA TTA TGC GGC CGC GAA TTA 75 Trp Met Glu Glu Val Ile Lys Leu Cys Gly Arg Glu Leu 20 **B-chain relaxin** GTT CGC GCG CAG ATT GCC ATT TGC GGC ATG AGC ACC TGG 114 Val Arg Ala Gln Ile Ala Ile Cys Gly Met Ser Thr Trp 35 30 AAA AGG AAA CCC ACT GGT TAT GGT TCT CGA AAA AAG 153 AGC Lys Arg Lys Pro Thr Gly Tyr Gly Ser Arg Lys Lys 50 45 40 Synthetic DNA Encoding C-Pentide CAA CTC TAC AGT GCA TTG GCT AAT AAA TGT TGC CAT 192 AGA Gln Leu Tyr Ser Ala Leu Ala Asn Lys Cys Cys His 60 55 A-chain relaxin GTT GGT TGT ACC AAA AGA TCT CTT GCT AGA TTT TGC 228 Val Gly Cys Thr Lys Arg Ser Leu Ala Arg Phe Cys 70 65











AlaProGlnThrProArgProValAlaGluIleValProSerPheIleAsnLysAspThrGluThrIleAsnMetMetSerGCTCCTCAGACACCTAGACCAGTGGCAGAAATTGTGCCATCCTTCATCAACAAAGATACAGAAACCATAAATATGATGTC

AspSerSerProSerGluLeuLysTyrLeuGlyLeuAspThrHisSerArgLysLysArgGlnLeuTyrSerAlaLeuAla GACAGCAGTCCTTCAGAATTAAAATACTTAGGCTTGGATACTCTTCTCGAAAAAAAGAGACAACTCTACAGTGCATTGGC

FIG. 9A

aaaagaagaagagtcgaaagaactgtgtgcgcaggtagaagctttggagattatcgtcactgcaatgcttcgcaatatg GCGCAAAATGACCAACAGCGGTTGATTAGATCAGGTAGAGGGGGGGCTGTACGAGGTAAAGCCCGATGCCAGTTCCTGA CSACGATACGGAGCTGCTGCGCGATTACGTAAAGAAGTTAITGAAGCATCCTCGTCAGTAAAAAGTTAATCTTTTCAACA gctgtcataaagttgtcacggcccgagacttatagtcgctttgttttttatttttaatgtacgcaagttcacgta gaattcaacttctccatactttggataaggaaatacagacatgaaaatctcattgctgagttgttatttaagcttgccc

-23 Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met ATG AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG **AAAAGGGTATCTAGAGGTTGAGGTGATTTT** STII S.D. tro S.D.

The val phe ser ile Ala Thr Ash Ala Tyr Ala Asp Ser Trp Met Glu Glu Val Ile Lys rtc Gtt Ttr Tct Atr Gct Aca Ant Gcc Taf Gca Gac Tca TGG atg Gag Gra Gtt Atr Ara

10 Leu Cys Gly Arg Glu Leu Val Arg Ala Gln Ile Ala Ile Cys Gly Met Ser Thr Trp Ser TTA TGC GGC CGC GAA TTG GTA CGC GCG CAA ATA GCG ATA TGC GGT ATG AGT ACA TGG AGT

TGAAGAA

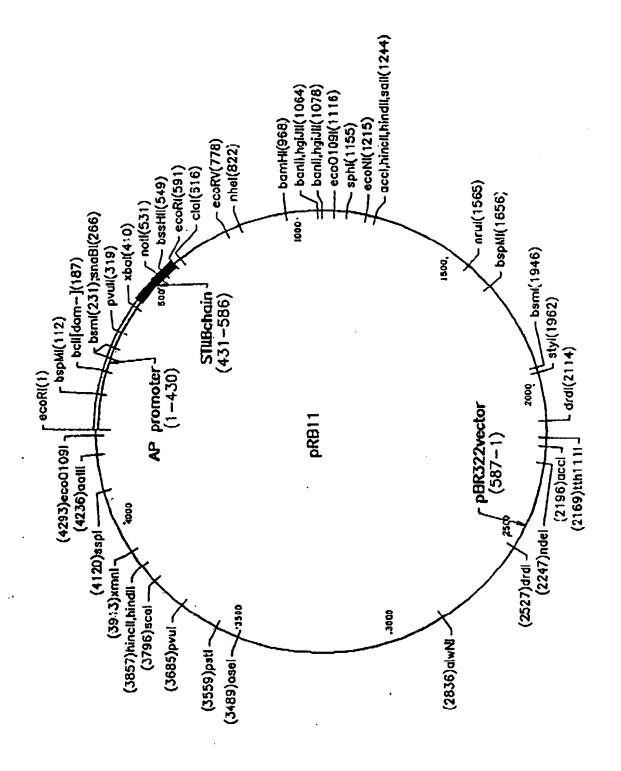
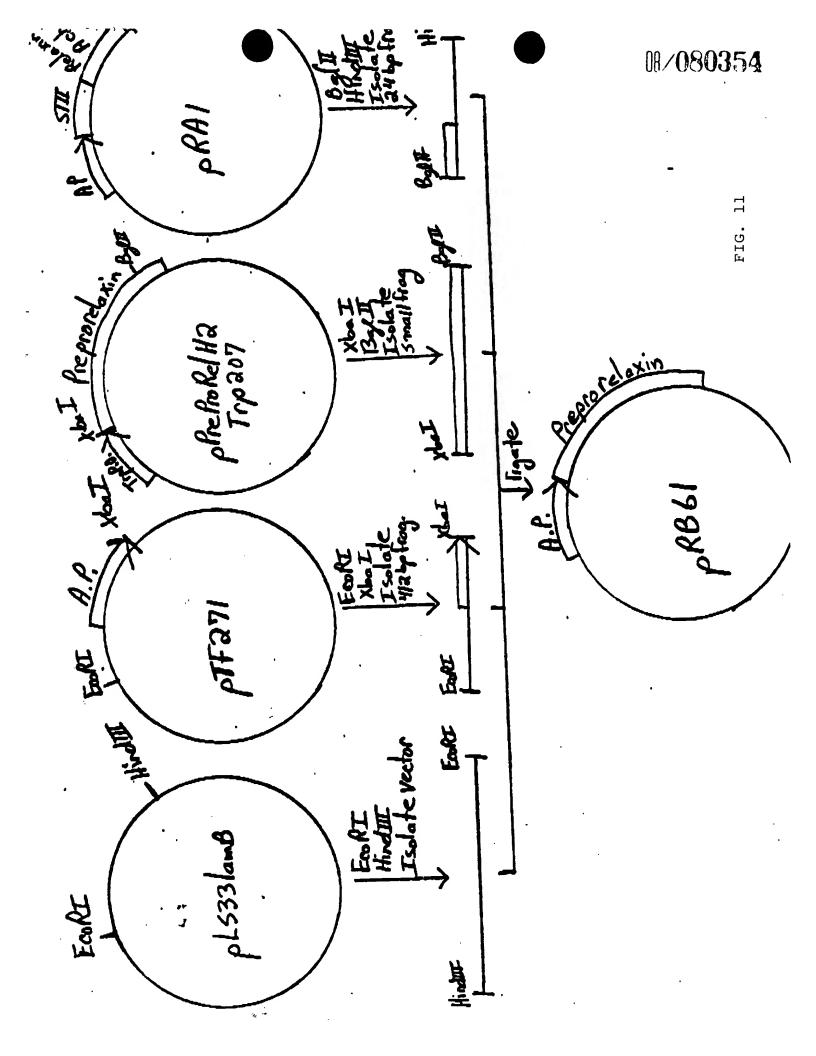
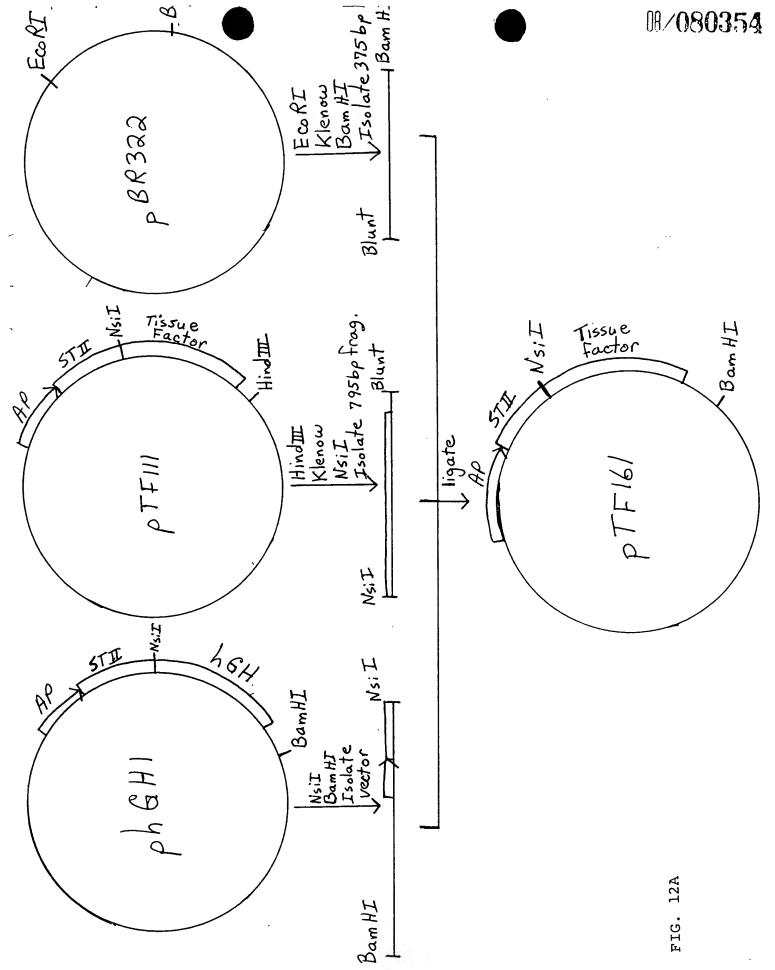


FIG. 10A





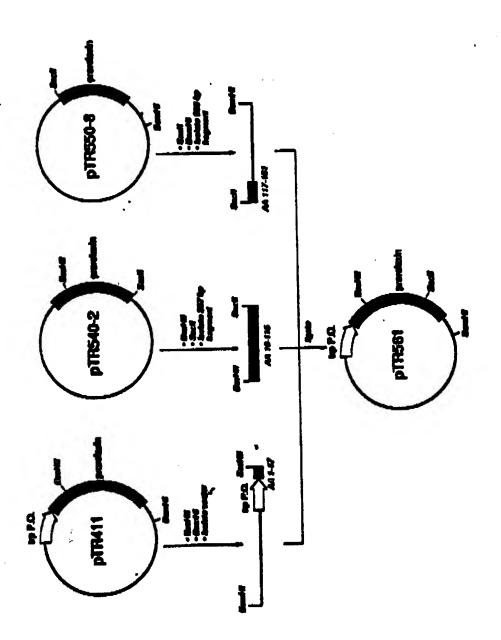
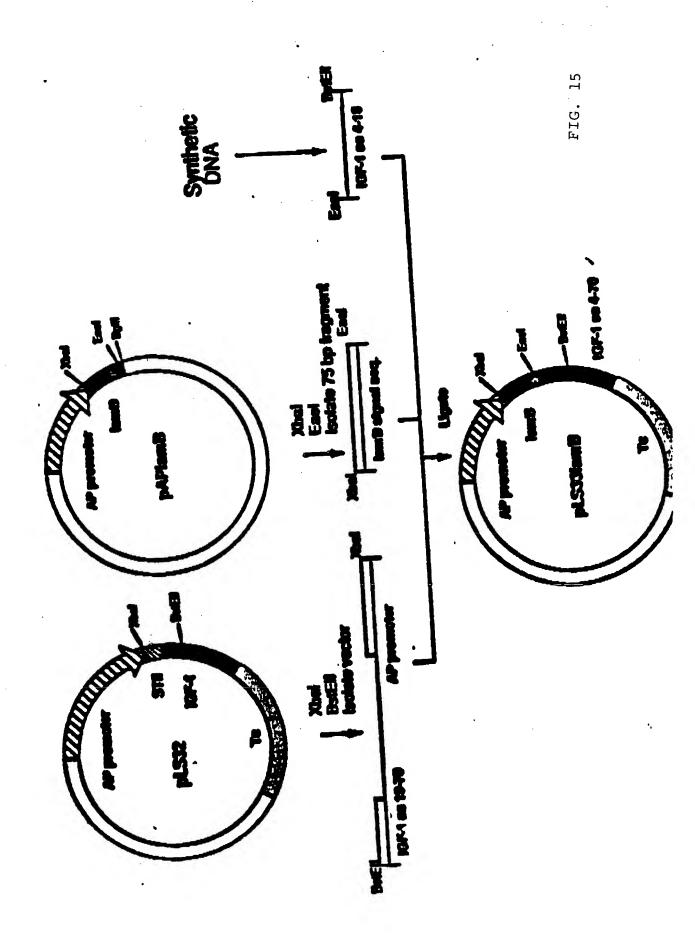


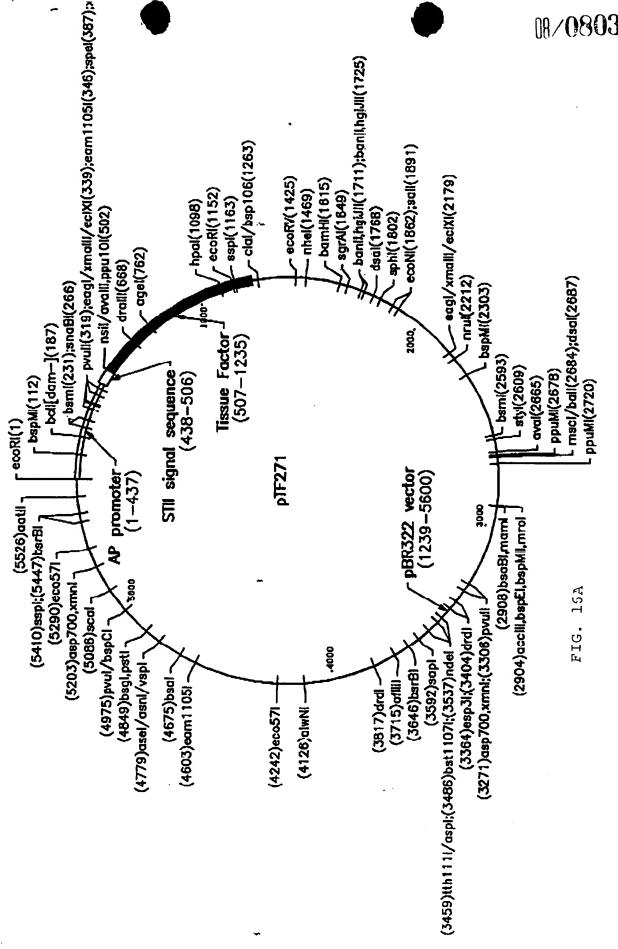
FIG. 14

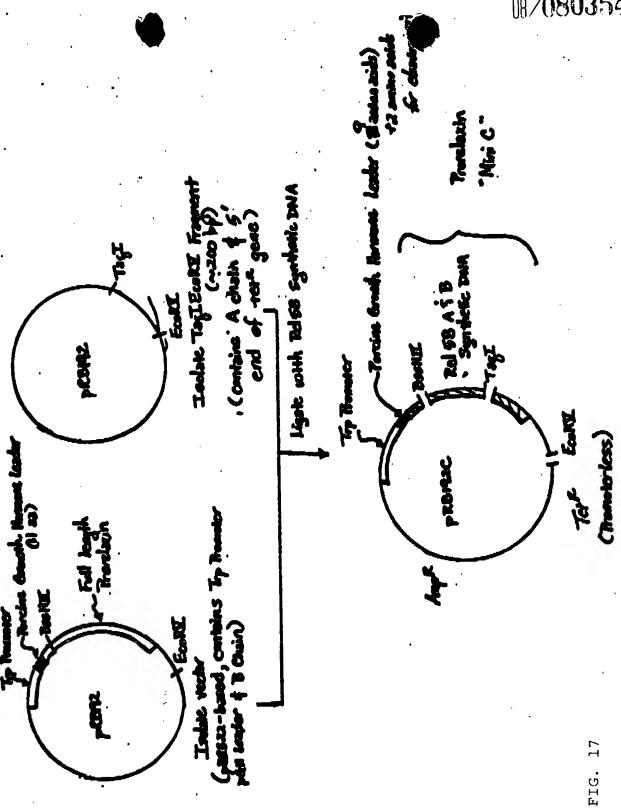


GAATTCAACTTCTCCATACTTTGGATAAGGAAATACAGACATGAAAAATCTCATTGCTGAGTTGTTATTTAAGCTTGCCC
AAAAAGAAGAAGAAGTCGAAAGAACTGTGTGCGCAGGTAGAAGCTTTGGAGATTATCGTCACTGCAATGCTTCGCAATATG
GCGCAAAATGACCAACAGCGGTTGATTGATCAGGTAGAGGGGGGGCGCTGTACGAGGTAAAGCCCGATGCCAGCATTCCTGA
CGACGATACGGAGCTGCTGCGCGGATTACGTAAAGAAGTTATTGAAGCATCCTCGTCAGTAAAAAGTTAATCTTTTCAACA
GCTGTCATAAAGTTGTCACGGCCGAGACTTATAGTCGCTTTGTTTTTATTTTTTAATGTATTTGTAACTAGTACGCAAGT

-23 tro S.D. STII S.D. Met Lys Lys Asn Ile Ala Phe Leu Leu Ala ATG AAA AAG AAT ATC GCA TIT CTT CTT GCA TCACGTAAAAAGGGTATCTAGAGGTTGAGGTGATTTT Ser Met Phe Val Phe Ser lie Ala Thr Asn Ala Tyr Ala Ser Gly Thr Thr Asn Thr Val TCT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GCA TCA GGC ACT ACA AAT ACT GTG Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro GCA GCA TAT AAT TTA ACT TGG AAA TCA ACT AAT TTC AAG ACA ATT TTG GAG TGG GAA CCC Lys Pro Val Asn Gin Val Tyr Thr Val Gin Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser AAA CCC GTC AAT CAA GTC TAC ACT GTT CAA ATA AGC ACT AAG TCA GGA GAT TGG AAA AGC Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val AAA TGC TTT TAC ACA ACA GAC ACA GAG TGT GAC CTC ACC GAC GAG ATT GTG AAG GAT GTG Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly AAG CAG ACG TAC TTG GCA CGG GTC TTC TCC TAC CCG GCA GGG AAT GTG GAG AGC ACC GGT Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn TCT GCT GGG GAG CCT CTG TAT GAG AAC TCC CCA GAG TTC AGA CCT TAC CTG GAG ACA AAC 120 Leu Gly Gin Pro Thr Ile Gin Ser Phe Giu Gin Val Gly Thr Lye Val Asn Val Thr Val CTC GGA CAG CCA ACA ATT CAG AGT TTT GAA CAG GTG GGA ACA AAA GTG AAT GTG ACC GTA Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe GAA GAT GAA CGG ACT TTA GTC AGA AGG AAC ACT TTC CTA AGC CTC CGG GAT GTT TTT Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr GGC AAG GAC TTA ATT TAT ACA CTT TAT TAT TGG AAA TCT TCA AGT TCA GGA AAG AAA ACA Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe GCC AAA ACA AAC ACT AAT GAG TTT TTG ATT GAT GTG GAT AAA GGA GAA AAC TAC TGT TTC Ser Val Glo Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val AGT GTT CAA GCA GTG ATT CCC TCC CGA ACA GTT AAC CGG AAG AGT ACA GAC AGC CCG GTA 220 Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Ile Phe Tyr Ile Ile Gly Ala Val GAG TGT ATG GGC CAG GAG AAA GGG GAA TTC AGA GAA ATA TTC TAC ATT GGA GCT GTG Val Phe Val Val Ile Ile Leu Val Ile Ile Leu Ala Ile Ser Leu His OC\* GTA TTT GTG GTC ATC CTT GTC ATC CTG GCT ATA TCT CTA CAC TAA AATTCTCATGT

TTGACAGCTTATCATCGATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGTATGA
AATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGCATAGGCTTGGTTATGCCGGTACTG
CCGGGCCTCTTGCGGGGATATCGTCCATTCCGACAGTATCGCCAGTCACTATGGCGTGCTGCTAGCGCTATATGCGTTGAT
GCAATTTCTAT









MetLysSęrAsnAsnAlaLeuIleValIleLeuGlyThrValThrLeuAspAlaValGlyIleGlyLeuValMet AAGCTTATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGCATAGGCTTGGTTAT  $\tt ProValLeuProGlyLeuLeuArgAspIleValHisSerAspSerIleAlaSerHisTyrGlyValLeuLeuAlaLeuTyr$ GCCGGTACTGCCGGGCTCTTGCGGGATATCGTCCATTCCGACAGCATCGCCAGTCACTATGGCGTGCTGCTAGCGCTAT

AlaLeuMetGlnPheLeuCysAlaProValLeuGlyAlaLeuSerAspArgPheGlyArgArgProValLeuLeuAla ArgcGrTGArGCAATTTCTATGCGCACCCGTTCTCGGAGCACTGTCCGACCGCTTTGGCCGCCGCCCAGTCCTGCTCGCT TCGCTACTTGGAGCCACTATCGACTACGCGATCATGGCGACCACCCCGTCCTGTGGATCC SerLeuLeuGlyAlaThrIleAspTyrAlaIleMetAlaThrThrProValLeuTrp

18A FIG.

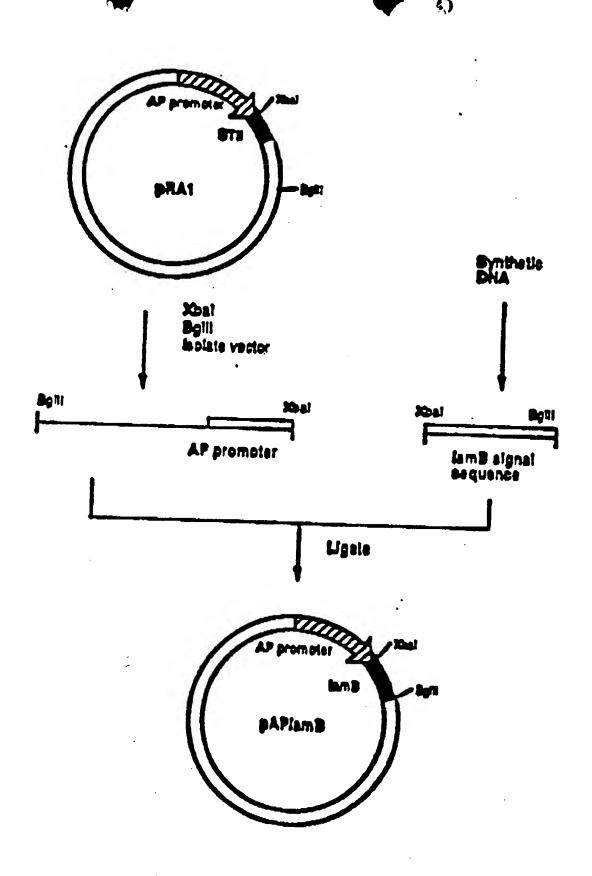


FIG. 19